

ride is purified for use in therapeutic or nutritional products, or the bacteria are used directly in such products.

[0009] The bacterium also comprises a functional β -galactosidase gene. The β -galactosidase gene is an endogenous β -galactosidase gene or an exogenous β -galactosidase gene. For example, the β -galactosidase gene comprises an *E. coli* lacZ gene (e.g., GenBank Accession Number V00296 (GI: 41901), incorporated herein by reference). The bacterium accumulates an increased intracellular lactose pool, and produces a low level of β -galactosidase.

[0010] A functional lactose permease gene is also present in the bacterium. The lactose permease gene is an endogenous lactose permease gene or an exogenous lactose permease gene. For example, the lactose permease gene comprises an *E. coli* lacY gene (e.g., GenBank Accession Number V00295 (GI:41897), incorporated herein by reference). Many bacteria possess the inherent ability to transport lactose from the growth medium into the cell, by utilizing a transport protein that is either a homolog of the *E. coli* lactose permease (e.g., as found in *Bacillus licheniformis*), or a transporter that is a member of the ubiquitous PTS sugar transport family (e.g., as found in *Lactobacillus casei* and *Lactobacillus rhamnosus*). For bacteria lacking an inherent ability to transport extracellular lactose into the cell cytoplasm, this ability is conferred by an exogenous lactose transporter gene (e.g., *E. coli* lacY) provided on recombinant DNA constructs, and supplied either on a plasmid expression vector or as exogenous genes integrated into the host chromosome.

[0011] The bacterium comprises an exogenous fucosyltransferase gene. For example, the exogenous fucosyltransferase gene encodes $\alpha(1,2)$ fucosyltransferase and/or $\alpha(1,3)$ fucosyltransferase. An exemplary $\alpha(1,2)$ fucosyltransferase gene is the wcfW gene from *Bacteroides fragilis* NCTC 9343 (SEQ ID NO: 4). An exemplary $\alpha(1,3)$ fucosyltransferase gene is the *Helicobacter pylori* 26695 futA gene. One example of the *Helicobacter pylori* futA gene is presented in GenBank Accession Number HV532291 (GI:365791177), incorporated herein by reference.

[0012] Alternatively, a method for producing a fucosylated oligosaccharide by biosynthesis comprises the following steps: providing an enteric bacterium that comprises a functional β -galactosidase gene, an exogenous fucosyltransferase gene, a mutation in a colanic acid synthesis gene, and a functional lactose permease gene; culturing the bacterium in the presence of lactose; and retrieving a fucosylated oligosaccharide from the bacterium or from a culture supernatant of the bacterium.

[0013] To produce a fucosylated oligosaccharide by biosynthesis, the bacterium comprises a mutation in an endogenous colanic acid (a fucose-containing exopolysaccharide) synthesis gene. By “colanic acid synthesis gene” is meant a gene involved in a sequence of reactions, usually controlled and catalyzed by enzymes that result in the synthesis of colanic acid. Exemplary colanic acid synthesis genes include an rcsA gene (e.g., GenBank Accession Number M58003 (GI:1103316), incorporated herein by reference), an rcsB gene, (e.g., GenBank Accession Number E04821 (GI:2173017), incorporated herein by reference), a wcaJ gene, (e.g., GenBank Accession Number (amino acid) BAA15900 (GI:1736749), incorporated herein by reference), a wzxC gene, (e.g., GenBank Accession Number (amino acid) BAA15899 (GI:1736748), incorporated herein by reference), a wcaD gene, (e.g., GenBank Accession

Number (amino acid) BAE76573 (GI:85675202), incorporated herein by reference), a wza gene, (e.g., GenBank Accession Number (amino acid) BAE76576 (GI:85675205), incorporated herein by reference), a wzb gene, and (e.g., GenBank Accession Number (amino acid) BAE76575 (GI: 85675204), incorporated herein by reference), and a wzc gene (e.g., GenBank Accession Number (amino acid) BAA15913 (GI:1736763), incorporated herein by reference).

[0014] This is achieved through a number of genetic modifications of endogenous *E. coli* genes involved either directly in colanic acid precursor biosynthesis, or in overall control of the colanic acid synthetic regulon. Specifically, the ability of the host *E. coli* strain to synthesize colanic acid, an extracellular capsular polysaccharide, is eliminated by the deletion of the wcaJ gene, encoding the UDP-glucose lipid carrier transferase. In a wcaJ null background, GDP-fucose accumulates in the *E. coli* cytoplasm. Over-expression of a positive regulator protein, RcsA, in the colanic acid synthesis pathway results in an increase in intracellular GDP-fucose levels. Over-expression of an additional positive regulator of colanic acid biosynthesis, namely RcsB, is also utilized, either instead of or in addition to over-expression of RcsA, to increase intracellular GDP-fucose levels. Alternatively, colanic acid biosynthesis is increased following the introduction of a null mutation into the *E. coli* lon gene (e.g., GenBank Accession Number L20572 (GI: 304907), incorporated herein by reference). Lon is an adenosine-5'-triphosphate (ATP)-dependant intracellular protease that is responsible for degrading RcsA, mentioned above as a positive transcriptional regulator of colanic acid biosynthesis in *E. coli*. In a lon null background, RcsA is stabilized, RcsA levels increase, the genes responsible for GDP-fucose synthesis in *E. coli* are up-regulated, and intracellular GDP-fucose concentrations are enhanced.

[0015] For example, the bacterium further comprises a functional, wild-type *E. coli* lacZ⁺ gene inserted into an endogenous gene, for example the lon gene in *E. coli*. In this manner, the bacterium may comprise a mutation in a lon gene.

[0016] The bacterium also comprises a functional β -galactosidase gene. The β -galactosidase gene is an endogenous β -galactosidase gene or an exogenous β -galactosidase gene. For example, the β -galactosidase gene comprises an *E. coli* lacZ gene. The endogenous lacZ gene of the *E. coli* is deleted or functionally inactivated, but in such a way that expression of the downstream lactose permease (lacY) gene remains intact.

[0017] The bacterium comprises an exogenous fucosyltransferase gene. For example, the exogenous fucosyltransferase gene encodes $\alpha(1,2)$ fucosyltransferase and/or $\alpha(1,3)$ fucosyltransferase. An exemplary $\alpha(1,2)$ fucosyltransferase gene is the wcfW gene from *Bacteroides fragilis* NCTC 9343 (SEQ ID NO: 4). An exemplary $\alpha(1,3)$ fucosyltransferase gene is the *Helicobacter pylori* 26695 futA gene. One example of the *Helicobacter pylori* futA gene is presented in GenBank Accession Number HV532291 (GI:365791177), incorporated herein by reference.

[0018] A functional lactose permease gene is also present in the bacterium. The lactose permease gene is an endogenous lactose permease gene or an exogenous lactose permease gene. For example, the lactose permease gene comprises an *E. coli* lacY gene.